Treatment of adjuvant arthritis with antibradykinin drugs

The discovery of the polypeptides of the bradykinin type has made possible an additional approach to the problem of mediation of inflammatory reaction. Besides such mediators as histamine and 5-hydroxytryptamine, bradykinin could be responsible for some syndromes of inflammation particularly for the pathogenesis of the delay phase of the reactions (Turk & Willoughby, 1969).

We now present the results of experiments on bradykininogen and the influence of some antibradykinin drugs on the course of rheumatic-like pathology in rats. Both the induction procedure and the gradation of its course were described by Pelczarska (1969).

To estimate the level of bradykininogen in blood plasma in adjuvant-treated rats, seven groups of eight randomly bred Wistar rats were used. The animals were injected with complete Freund adjuvant and at appropriate intervals each group of animals was bled. Bradykininogen levels were estimated according to Diniz, Carvalho & others (1961).

The following pattern of examination was used. Group I: animals in the induction phase of immunological response (24 h after injection of adjuvant); Group II: animals in the latent period of the condition (7th day); Groups III–V: rats in the period of full manifestation of the condition (17th, 22nd, and 30th day, respectively); Group VI: after recovery (40th day); Control group (non-arthritic rats). The results for bradykininogen are shown in Fig. 1A.

To test the therapeutic effects of anti-inflammatory drugs, possessing antibradykinin activity, adjuvant-treated rats of randomly bred Wistar strain were treated with indomethacin (1 mg/kg, orally), sodium phenylbutazone (8 mg/kg, i.p.), amidopyrine (3 mg/kg, i.p.) and sodium salicylate (65 mg/kg, orally). The drugs were administered one day before Freund adjuvant and then three times weekly. The results are in Fig. 1B.

Antibradykinin activity of the drugs was tested both *in vivo* and *in vitro*.

The *in vivo* experiments were made on inbred August rats. The animals had been pretreated as described above with daily doses of the drugs for 3 days before experiments started (on the 3rd day, 1 h before testing). The pretreated and control

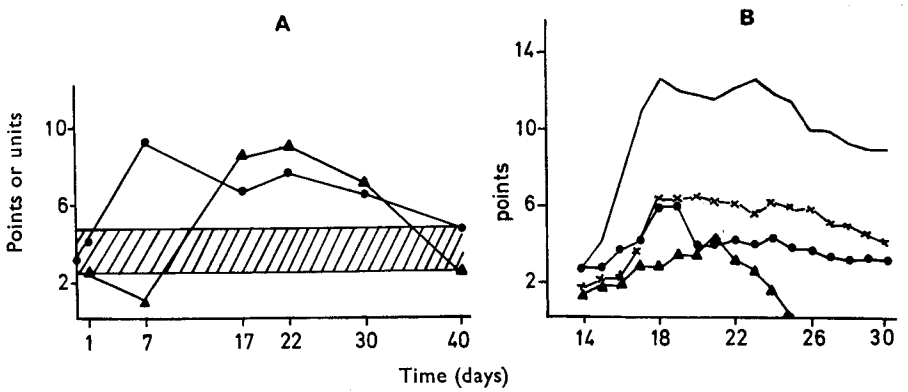

FIG. 1A. Average mean of bradykininogen concentration in blood plasma of arthritic rats (—▲—) in units of bradykinin (ordinate) formed per ml of plasma. Intensity of arthritic manifestation (—●—) as scored according to a conventional 18 point pattern (ordinate) (average mean in points see Giełdanowski, Pelczarska & others, 1969). Hatched area is control level of bradykininogen.

B. Influence of drugs on the course of adjuvant arthritis. —●— Indomethacin; —×— Phenylbutazone; —▲— Amidopyrine; —— Control. Ordinate: points as in Fig. 1A.

Table 1. *Influence of drugs on skin reaction to bradykinin and their antibradykinin activity* in vitro. Skin reaction to bradykinin was assessed on the abdomen surface of prepared skin flaps as follows: the area of brown coloured patch (in cm^2) was multiplied by the number of points (1–4 according to the intensity of the skin reaction). Average means. The concentration of the drugs in the *in vitro* test are relative to the equivalent of LD50 of indomethacin, the therapeutic concentration of which was assumed to be 10 μg/ml

Drugs	Skin reaction (inhibition %)	*In vitro* test	
		Concentration of drugs (μg/ml)	Inhibition (%)
Indomethacin	68	10	0
Phenylbutazone ..	67	3	0
Amidopyrine	93	3·3	30
Sodium salicylate ..	59	0·05	12

rats were then injected intradermally in the skin of the abdomen with 0·1 ml of bradykinin (100 μg/ml) and in the other flank with 0·1 ml of physiological solution of saline. Simultaneously the animals received intravenously 1% colloidal silver (1 ml/100 g) for the visualization of the inflammatory region in the skin (Jancsó, 1961).

The animals were killed 3 h later and the skin reaction to bradykinin was scored (Table 1).

Bradykininogen concentrations in blood plasma increased markedly, particularly in the latent period of the disease (Fig. 1). The means of bradykininogen values during the period of full manifestation of the inflammatory condition were about twice as high as normal and then (on the 40th day) returned to the threshold value.

Houssay, Monfort & others (1964) had found an increase of globulin and diminution of the albumin fraction of plasma proteins as the condition developed. Again, Lowe (1964) reported the appearance of a factor migrating (in immune-electrophoresis) near α_2-globulin fraction at the period of between 16–20th day after the injection of adjuvant. So the question arises whether the augmentation of quantity of the bradykinin precursor in plasma is really significant in the pathogenesis of the adjuvant induced condition.

All the drugs tested showed significant antibradykinin activity in the skin reaction test, and amidopyrine and to some degree sodium salicylate in the *in vitro* test.

But among the drugs, indomethacin, amidopyrine (in doses of 1/75 of the LD50) and phenylbutazone (in doses of 1/25 LD50), showed favourable effects upon the arthritis syndrome in the adjuvant-treated rats. Sodium salicylate did not influence the course of the condition.

The results of the experiments indicate that bradykinin can play a role in the inflammatory process, but antibradykinin properties of drugs need not be correlated with their anti-arthritic activity, as was shown with sodium salicylate.

The increase of bradykininogen level in blood plasma seems to have a significance in the pathology of adjuvant arthritis influenced by intracutaneous injection of complete Freund adjuvant in rats.

Our thanks are due to Miss J. Lewandowska for her skilful technical assistance.

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February 27, 1970

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Inhibitory effect of *p*-hydroxyphenylisopropylarterenol on the isolated human myometrium

There is a need for drugs to suppress excessive uterine activity of premature labour. Compounds with β-adrenergic receptor stimulating properties have been synthesized and tested for this action *in vitro* and *in vivo*. One of the most active seems to be *p*-hydroxyphenylisopropylarterenol (Cc 25; Philips-Duphar). In this paper two characteristics not previously described are presented*.

Myometrial strips from patients undergoing hysterectomy, legal abortion or Caesarean section, were mounted in an isolated organ bath and the motility recorded on a smoked drum (Bygdeman & Eliasson, 1963). The drug was dissolved in normal saline and fresh solutions were prepared immediately before use because at neutral pH there was a rapid auto-oxidation.

Myometrial strips (n = 50) at late proliferatory phase from 16 non-pregnant patients showed a clear inhibition (50% or more) of the amplitude or frequency of the contractions, or both, with the drug at $1–2{\cdot}5 \times 10^{-7}$ g/ml. When the spontaneous activity had been restored after washing, the myometrium was always completely refractory to a second dose, even if this was 10 times larger than the first (Fig. 1). A subsequent dose of PGE_1 always inhibited the motility indicating a normal reactivity to other inhibitors.

Myometrial strips (n = 16) from four patients in the 12th to 20th week of gestation responded qualitatively in the same way as those from the non-pregnant patients but the sensitivity was 100–1000 times higher, i.e. a clear inhibition could be obtained with $0{\cdot}1–1 \times 10^{-9}$ g/ml. The tachyphylaxis was not as complete as for the non-pregnant myometrium (Fig. 2).

Myometrial strips from patients at term were less sensitive to the drug than those from non-pregnant women. In one experiment (three strips from one patient) a clear inhibition was obtained with $0{\cdot}1 \times 10^{-6}$ g/ml, while in two experiments (five strips) no effect was noted with $0{\cdot}25–0{\cdot}75 \times 10^{-6}$ g/ml. Doses up to 1×10^{-5} g/ml were tested, but were always without effect. Whether this arose from primary insensitivity or tachyphylaxis could not be ascertained.

Propranolol (10^{-5} g/ml) completely blocked the effect of the drug.

The effects of the drug on the human uterus *in vitro* have also been described by

* Presented at an International Symposium on Uterine Physiology and Pharmacology, June 20–22, 1968, New York.